Physicochemical Evaluation of Bioactive Polymeric Composites Based on Hybrid Amorphous Calcium Phosphates

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Abstract: Amorphous calcium phosphate (ACP)-filled methacrylate composites were recently found to effectively remineralize *in vitro* caries-like enamel lesions. Their inferior mechanical properties compared to glass-filled composites, however, limit their use as a dental restorative material. In this study, the feasibility of introducing glass-forming elements (tetraethoxysilane or zirconyl chloride) during the low-temperature synthesis of ACP was investigated. Composites based on such hybrid fillers (mass fraction, 40%) were evaluated to establish whether hybridization strengthened the composites via improved interfacial interactions with the polymer phase without compromising the release of the mineral ions. Two types of visible-light cured resins were prepared: BTHZ resin from 2,2-bis[p-(2'-hydroxy-3'-methacryloxypropoxy)phenyl]propane (BisGMA), triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA) and zirconyl methacrylate (ZrM), and TP resin from TEGDMA and pyromellitic glycerol dimethacrylate (PMGDM). Hybridized fillers and BTHZ- and TP-based composites were characterized by the IR spectroscopy, X-ray diffraction, dissolution/transformation kinetic studies, and biaxial flexure strength (BFS) testing before and after immersion in buffered saline solutions. The feasibility of improving the BFS via hybridization, while retaining, if not enhancing the remineralizing potential was demonstrated for BTHZ-based composites. Both BFS and remineralizing ability of the TP-composites, however, deteriorated upon their exposure to an aqueous environment. Therefore, hybridized ACP-filled BTHZ composites have a potential for utilization in more demanding restorative, sealant, and adhesive applications. © 2000 John Wiley & Sons, Inc.* J Biomed Mater Res (Appl Biomater) 53: 381–391, 2000

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INTRODUCTION

Over the broad range of solution conditions in which spontaneous precipitation of calcium phosphates (CaPs) occurs, amorphous calcium phosphate (ACP) is the first phase to form. It precedes the appearance of hydroxyapatite (HAP), the final, thermodynamically stable product in precipitation from neutral or basic solutions. ACP is unique among the CaPs in that it lacks the long-range, periodic atomic scale order of crystalline CaPs. Although the uniqueness of ACP has been a subject of considerable interest,[1,2] the instability of ACP in solution is of greater relevance to understanding the dynamics of HAP formation by spontaneous precipitation. The relatively high solubility of ACP and its conversion to HAP in aqueous media might pose limitations for its use in applications where structural, mechanical, and chemical stabilities are desired. However, these same properties may make ACP suitable as a potential remineralizing agent in dental applications.

We have recently developed an experimental class of bioactive composites based on a polymer matrix phase derived from ambient polymerization of acrylic monomers and a filler phase consisting of ACP.[3,4] When the ACP was stabilized by ions that retard its conversion to HAP (primarily

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TABLE I. Methacrylate Monomers Employed for Resin Formulations

Acronym	Monomer	Manufacturer
BisGMA	2,2-bis[*p*-(2′-hydroxy-3′-methacryloxy propoxy)phenyl]propane	Freeman Chemical Corp. Port Washington; WI
HEMA	2-hydroxyethyl methacrylate	Rohm Tech, Inc.; Malden; MA
PMGDM	pyromellitic glycerol dimethacrylate	Esschem, Essington; PA
TEGDMA	triethylene glycol dimethacrylate	Esschem, Essington; PA
ZrM	zirconyl methacrylate	Rohm Tech, Inc.; Malden; MA

pyrophosphate $P_2O_7^{4-}$, and to the lesser extent Mg^{2+}), it was possible to take advantage of ACP's solubility and obtain substantial release of Ca^{2+} and PO_4 ions. Moreover, the solution levels of Ca^{2+} and PO_4 attained from these releases were sustainable for sufficient periods of time to promote the recovery of mineral-deficient tooth structures in *in vitro* situations.[5] Moreover, it has been shown that this remineralization could be accomplished with only a small portion of theoretically available mineral ions actually being released into the external media. However, in contrast to conventional glass composites, such bioactive composites are relatively weak,[6] because ACP powders do not act as reinforcing fillers as is the case with silanized glass fillers.

The objective of this study was to determine if the introduction of glass-forming elements during the low-temperature preparation of $P_2O_7^{4-}$-stabilized ACP would result in hybrid fillers with greater potential for strengthening composites without adversely affecting their remineralizing ability. In particular, the feasibility of preparing silica- or zirconia-modified ACP by adding different levels of the hybridizing agents tetraethoxysilane (TEOS) or zirconyl chloride ($ZrOCl_2$), respectively, during the ACP synthesis was assessed. These hybrid ACP fillers were characterized physicochemically and then combined with two differently formulated methacrylate resin mixtures. The resulting composites were evaluated for their mechanical and remineralizing properties.

MATERIALS AND METHODS

Synthesis and Characterization of Hybrid ACP Fillers

Synthesis of hybrid $P_2O_7^{4-}$-stabilized ACP fillers employed a modified version of the ACP preparation protocol of Eanes et al.[7] ACP was spontaneously precipitated in a closed system (under CO_2-free N_2 in order to minimize CO_2 adsorption) at 22°C by rapidly mixing equal volumes of a 800 mmol/L $Ca(NO_3)_2$ solution (Ca reactant) and a 536 mmol/L Na_2HPO_4 solution that contained 10.72 mmol/L $Na_4P_2O_7$ (PO_4 reactant). The PO_4 reactant was brought to pH 12.5 with 1 mol/L NaOH prior to mixing. Appropriate amounts of hybridizing agent [tetraethoxysilane, $(C_2H_5O)_4Si$ — TEOS or zirconyl chloride ($ZrOCl_2$)] were added to the Ca reactant to achieve TEOS:$Ca(NO_3)_2$ or $ZrOCl_2$:$Ca(NO_3)_2$ mol ratios of 0.05, 0.10, or 0.20. The acronyms 5T, 10T, 20T and 5Zr, 10Zr, 20Zr are used throughout the text to designate the different levels of hybridizer used in the filler synthesis (T stands for TEOS and Zr for $ZrOCl_2$). A mixture with mass fractions of 10% TEOS, 10% ethanol, 10% tartaric acid, and 70% water was proven to effectively prevent premature TEOS gelation and was used to introduce the TEOS during hybridization. After stabilization of the reaction pH at 10.5–11.0, which took less than 5 min, the suspension was filtered, and the solid phase washed with ice-cold ammoniated water and lyophilized. After completion of the isolation and drying procedures, the ACP fillers were kept in a desiccator until used for the preparation and physicochemical evaluation of composite disk specimens.

The lack of long-range periodic structure in the lyophilized hybrid ACP fillers, i.e., their being in an amorphous state, was verified by powder X-ray diffractometry (XRD). XRD profiles of the samples were recorded in the 4–60° 2θ range with CuKα radiation ($\lambda = 0.154$ nm) using a Rigaku X-ray diffractometer (Rigaku Denki Co., Ltd., Danvers, MA) operating at 40 kV and 40 mA. The samples were step-scanned in intervals of 0.010° 2θ at a scanning speed of 2.000 deg/min. The local (short-range) phosphate environment of the solids was evaluated by recording their FTIR spectra (KBr pellet technique: 2 mg solid per 400 mg KBr; 4000–400 cm^{-1}) using a Nicolet–Magma–IR FTIR System 550 spectrophotometer (Nicolet Instrument Corp., Madison, WI) purged with dry CO_2-free air.

The Ca/PO_4 ratio of the solids, after dissolution in HCl, was calculated from solution Ca^{2+} values (determined by atomic absorption spectroscopy at 422.7 nm using an air-acetylene flame; spectrophotometer #603, Perkin–Elmer, Norwalk, CT) and from solution PO_4 values (PO_4 determined as a blue molybdato complex[8] using a Cary #219 spectrophotometer (Varian Analytical Instruments, Palo Alto, CA) tuned to an absorption wavelength of 882 nm. Atomic absorption spectroscopy using a nitrous oxide-acetylene flame was employed to determine the amount of the Si (absorption at 251.6 nm) and Zr (absorption at 360.1 nm) incorporated in hybrid fillers. Both Si and Zr samples, as well as the standard solutions, contained a small amount of HF to ensure complete dissolution.

The specific surface area of the fillers (expressed as m^2/g; triplicate measurements for each sample) was determined by the triple point BET method[9] using the Quantasorb sorption system (Quantachrome Corp., Greenvale, NY).

Resin Formulations

Methacrylate resins used in this study were formulated from the commercial monomers listed in Table I. The components

TABLE II. Components of the Photoinitiation Systems

Acronym	Component	Manufacturer
CQ	camphorquinone	Aldrich Chem. Co.; Milwaukee, WI
4625 DAROCUR	2-hydroxy-2-methyl-1-phenyl-1-propanone & diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide	Ciba-Geigy Corp.; Hawthorne, NY
4EDMAB	Ethyl-4-*N*,*N*-dimethyl aminobenzoate	Aldrich Chem. Co.; Milwaukee, WI
369 IRGACURE	2-benzyl-2-(dimethylamino)-1-(4-(4-morpholinyl) phenyl)-1-butanone	Ciba-Geigy Corp.; Hawthorne, NY

of the photo-initiation systems used for the photo-activation of the resins are provided in Table II. Resin compositions (% of the components on a mass fraction basis) are given in Table III. The **BTHZ** resin was made from the widely used dental monomers, 2,2-bis[p-(2'-hydroxy-3'-methacryloxypropoxy)phenyl]propane (**B**isGMA) and triethylene glycol dimethacrylate (**T**EGDMA), to which a substantial amount of 2-hydroxyethyl methacrylate (**H**EMA) was added to enhance the hydrophilicity of the resin after it is polymerized. Zirconyl methacrylate (**Z**rM) was included as a dispersing agent for the ACP filler. The **TP** resin consisted of equal amounts by mass of **T**EGDMA and pyromellitic glycerol dimethacrylate (**P**MGDM). PMGDM is a difunctional methacrylate that contains two carboxylic acid groups, which serve to increase the adhesiveness of TP-based composites to dentin structures.

Preparation and Evaluation of Composite Disk Specimens

The experimental protocol employed in the preparation, initial characterization, dissolution/immersion, and mechanical testing of the composite disks is outlined in Figure 1. Composite pastes were made up of hybrid ACP fillers (5T, 10T, 20T, 5Zr, 10Zr, 20Zr) with either BTHZ or TP resins in a resin/filler mass ratio = 1.5 (mass fractions of 60% and 40% for the resin and the filler, respectively). Unhybridized ACP-based BTHZ and TP composites with the same resin/filer mass ratio were prepared as control composites.

After the initial blending by hand spatulation, the homogenized pastes were kept under vacuum overnight to eliminate any air entrained during mixing. Before being molded into disks (flat Teflon molds: 1.12–1.49 mm in thickness, and 14.42–15.00 mm in diameter) uncured pastes were examined by XRD and FTIR to verify that no conversion of the amorphous ACP filler into HAP occurred at this stage. The circular opening of the mold was filled with the paste, each end of the paste-filled mold was covered by a mylar film and a glass slide, and the assembly was clamped together with a spring clip. The disks were photocured with visible light (2 min/each face of the mold; Triad 2000, Dentsply International Inc., York, PA) and then postcured at 37°C for 24 h in air. Disk specimens were then characterized by XRD and FTIR. An appropriate number of the disk specimens (dry composite samples) in each experimental group was sacrificed at this step in the protocol for biaxial flexure strength (BFS) testing.

TABLE III. Composition of the Resins Used for the Preparation of Composites (Mass Fraction, %)

Monomer/Component	BTHZ Resin	TP Resin
BisGMA	35.10	—
HEMA	28.00	—
PMGDM	—	48.65
TEGDMA	35.10	48.65
ZrM	0.80	—
CQ	0.20	0.40
4625 DAROCUR	—	0.80
4EDMAB	0.80	—
369 IRGACURE	—	1.50

The remineralizing ability of the composites was tested by immersion (dissolution) experiments. Each individual disk specimen was held by means of stainless steel wire frames in 100 mL of a NaCl solution [HEPES-buffered (pH = 7.40), ionic strength 0.13 mol/L, 37°C, continuous magnetic stirring] for at least 500 h. Aliquots were taken at predetermined time intervals, filtered (Millex GS assemblies; Millipore, Bedford, MA), and the filtrates analyzed for Ca^{2+} and total phosphate (PO_4) using atomic absorption spectroscopy (AAS) and UV/VIS spectrophotometry, respectively. Ca^{2+} and PO_4 filtrate data were normalized to 500 mm^2 of the total surface area[10] of the disk exposed to the immersion solution.

Upon completion of the immersion tests, the disks were removed, dried, evaluated by XRD and FTIR, and then broken under the BFS conditions (wet composite samples). Fractured disks (after the completion of BFS measurements) were ground by mortar and pestle and were used for powder XRD and FTIR (KBr pellet method) screening.

BFS testing of both dry and wet composite disk specimens was performed according to ASTM recommendations.[11] The test specimen rested on three symmetrically spaced points near its periphery. The force was applied at a prescribed constant rate (1 mm/min) in a compression mode (Instron 5500R, Instron Corp., Canton, MA) to the center of the disk through a cylindrical ram until the specimen broke. The load at failure, the dimensions and elastic constants of the specimen, and the radii of the support and the load were used to compute the maximum tensile stress at the point of origin of the fracture (usually at the center of the tension surface) using the following expression[11]:

$$\mathrm{BFS} = -0.2387P(X - Y)/t^2, \qquad (1)$$

Resin (BTHZ or TP; mass fraction of 60 %) + Filler (5T, 10T, 20T, 5Zr, 10Zr or 20Zr; mass fraction of 40 %)
(hand-blended into paste)

Overnight storage
(moderate vacuum to reduce porosity and oxygen inhibition)

Physicochemical characterization of the paste
(XRD, FTIR)

Preparation of composite disk specimen
(Teflon molds)

Visible light polymerization
(470 nm; 2 min/each side of the disk)

Storage of the disk specimen
(24 h in the air; 37°C)

Characterization of dry disk specimen
(XRD, FTIR, BFS)

Immersion experiments
(kinetics of the mineral ion release from the disks into continuously stirred HEPES-buffered NaCl solution (pH = 7.40; ionic strength = 0.13 mol/L); 37°C)

Characterization of wet disk specimen
(XRD, FTIR, BFS)

Figure 1. Schema of the experimental steps employed in physicochemical and mechanical evaluation of the composites.

where *BFS* is the failure stress (MN/m^2 = MPa), P is the load at failure (N), $X = (1 + \nu)\ln(B/C)^2 + [(1 - \nu)/2](B/C)^2$, $Y = (1 + \nu)[1 + \ln(A/C)^2] + (1 - \nu)(A/C)^2$, ν is the Poisson's ratio ($\nu = 0.23$ taken for all calculations), A is the radius of the support circle (mm), B is the radius of the piston applying the load at the surface of contact (mm), C is the radius of disk specimen (mm), and t is the thickness of disk specimen (mm).

Statistical Methodology

Separate multifactorial ANOVA (GraphPad Software Inc., San Diego, CA and SigmaStat, Jandel Scientific Software, San Rafael, CA) were performed on the following 4 datasets: Ca/P ratios, the specific surface areas of the fillers, mineral ion release, and the mechanical properties (*BFS*) of the composites. For the cases where overall statistically significant differences are found with ANOVA, further tests were performed to determine significant differences among specific groups using an appropriate multiple comparison procedure.

RESULTS

Characterization of Hybrid ACP Fillers

When compared to the unhybridized ACP, no observable differences in the structural features of hybridized fillers were found, regardless of the level of hybridizer initially introduced into the precipitating system, (XRD patterns; Fig. 2). FTIR absorbance spectra (Fig. 3) showed only two wide bands: ν_1 and ν_3 phosphate stretching in the region of (900–1200) cm^{-1} and ν_4 phosphate bending in the region of (500–630) cm^{-1}, both typical for noncrystalline calcium phosphate.

Based on the Ca^{2+} and PO_4 contents of the dissolved fillers (Table IV), unhybridized ACP, TEOS-hybridized ACP (at all three investigated levels) as well as Zr-hybridized ACP at the lowest level of hybridizer (i.e., mol fraction, 5%) had a practically constant Ca/P mol ratio of 1.459 ± 0.058 (Dunnett multiple comparison tests indicated no significant difference between these groups). However, significant increases in the Ca/P ratio were obtained for 10Zr and 20Zr ACPs. The Si content of TEOS-hybridized fillers ranged from mass fraction of 2.74–3.63% and could not be correlated with the amounts of TEOS initially employed in the ACP preparation. However, the Zr levels in $ZrOCl_2$-hybridized ACPs varied inversely with the amounts of hybridizer initially present.

The specific surface areas of all the hybridized solids (Table V) were, on average, twice that of unhybridized ACP (single factor ANOVA: $p < 0.0001$; 2-tail test: $p < 0.019$). Differences among hybridized ACP groups were found to be not significant ($p > 0.10$).

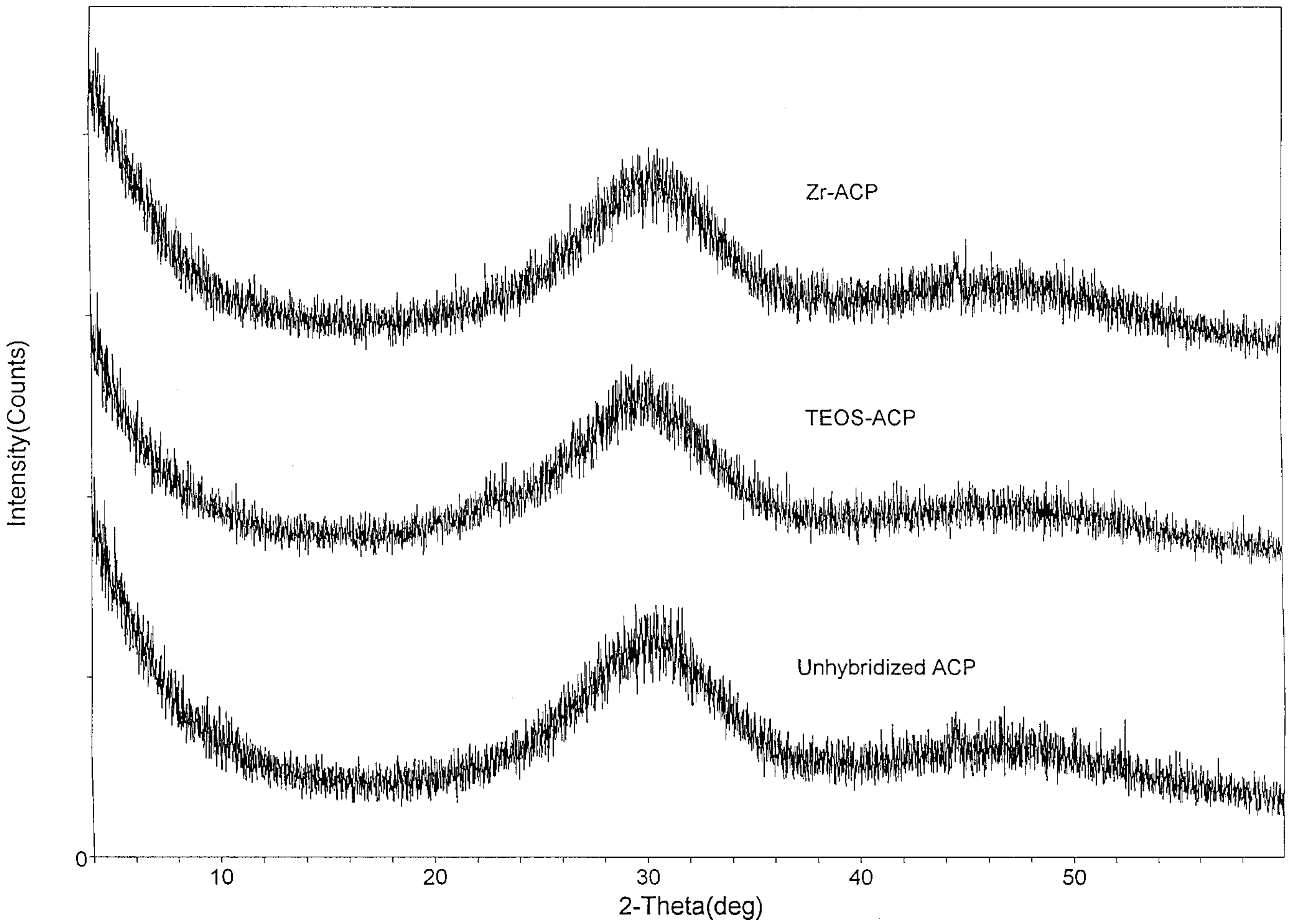

Figure 2. XRD patterns of the representative hybridized ACP fillers compared to unhybridized ACP.

Remineralizing Potential of Hybrid ACP-Filled BTHZ and TP Composites

Both unhybridized ACP- and hybridized ACP-based BTHZ composites steadily released mineral ions upon immersion in buffered saline until solution accumulations exceeded the saturation threshold for HAP. The kinetic profiles of unhybridized ACP and of 10T and 10Zr (representative of the hybrid ACP-based composites) are given in Figure 4. Statistical analysis (Dunnett multiple comparison test) of the maximum Ca^{2+} and PO_4 concentrations released from the disks after > 336 h of immersion (time when the plateau levels are reached; Table VI) showed no difference between unhybridized ACP and 5T, 10T, 20T, 5Zr, 10Zr, or 20Zr hybridized ACPs.

Upon immersion in saline buffer, internal conversion of the ACP filler into HAP occurred more slowly when hybridized ACPs were used. This slower conversion into HAP, a desirable property for ACP remineralizing composites because HAP is not sufficiently soluble to be effective as a remineralizing agent,[4] was evidenced by both XRD (data not shown) and FTIR (Fig. 5) analysis of composite disk specimens and was independent of the initial amount of hybridizer present. FTIR data (Fig. 5) obtained for a ground specimen of the unhybridized ACP composite upon the completion of the immersion clearly show three distinctive bands in the ν_1 and ν_3 region (approx. 1165 cm^{-1}, 1100 cm^{-1}, and 1033 cm^{-1}) and two bands in the ν_4 region (approx. 606 cm^{-1} and 562 cm^{-1}), all typical of the HAP structure. However, in the case of equally treated 10T- or 10Zr-hybridized ACP composites (chosen as representative hybridized composites), the characteristic HAP absorbance peaks are considerably weaker, which indicates that the hybridized ACP fillers utilized in these composites are still largely unconverted.

The solution profiles of Ca^{2+} and PO_4 ions released upon immersing disks composed of unhybridized ACP, 10T, and 10Zr-hybridized ACP and the TP resin in buffered saline are illustrated in Figures 6 and 7. Ca^{2+} concentrations (Fig. 6) released from 10T- and 10Zr-ACP composites after 74–96 h of immersion (0.944 ± 0.077 mmol/L and 1.17 (0.123 mmol/L, respectively) were significantly higher (p values of the unpaired t-test $= 0.012$ and < 0.0001, respectively) than the corresponding values obtained from similarly treated 10T- and 10Zr-BTHZ composites (0.766 ± 0.064 mmol/L and 0.695 ± 0.049 mmol/L, respectively). Once reached, these high Ca^{2+} levels, however, were not sustainable over extended time periods. Significant Ca-reincorporation by the

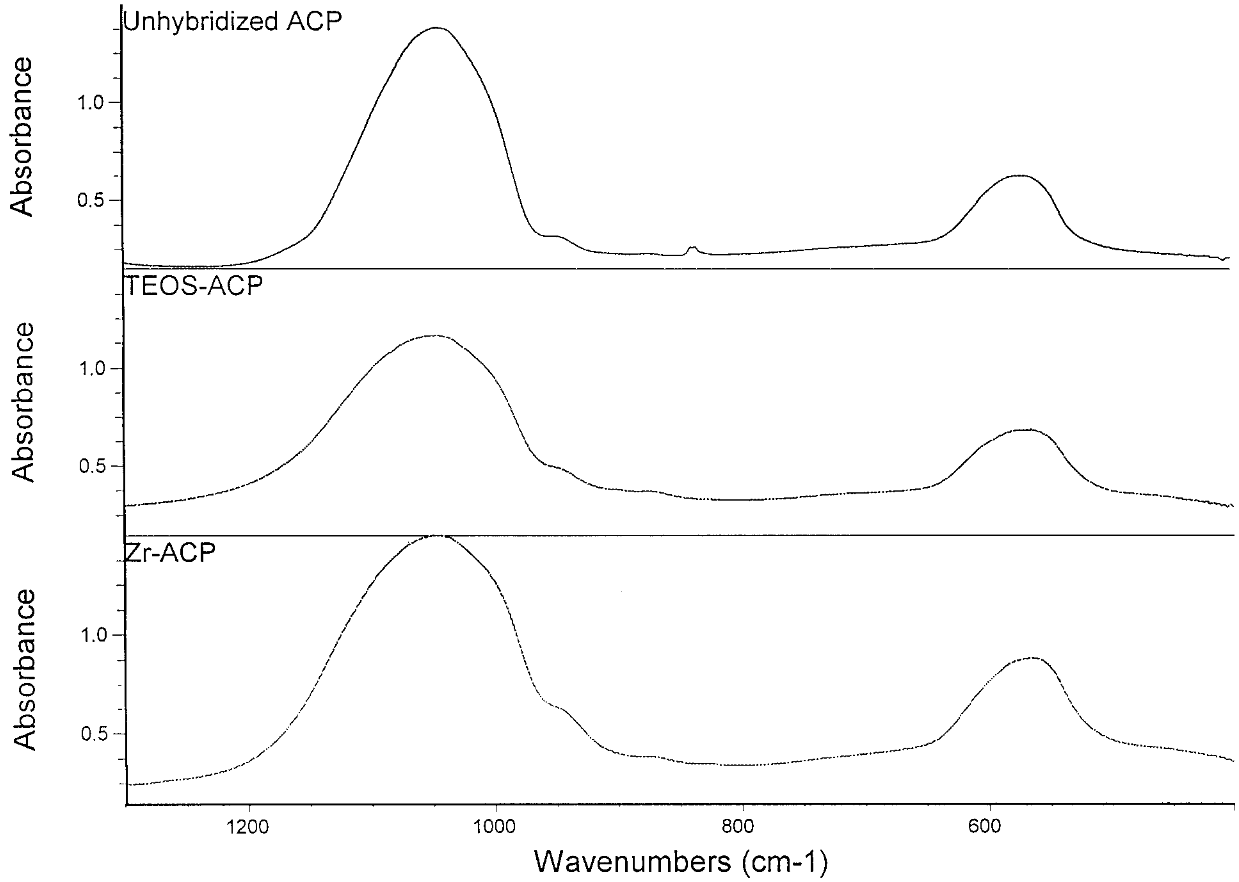

Figure 3. Typical FTIR spectra of hybridized ACP fillers compared to unhybridized ACP.

disk occurred between 100–150 h of immersion resulting in the reduction of the soluble (available) Ca^{2+} concentrations to only (0.30–0.35) mmol/L. A similar profile, i.e., a maximum Ca^{2+} concentration of (0.47 $\pm$ 0.04) mmol/L reached within 24 h followed by a steady decrease to undetectable levels by 160 h, was obtained for the unhybridized ACP composites. Unlike the Ca^{2+} release, PO_4 release continued to steadily rise during the immersion of unhybridized ACP composites (Fig. 7) to concentrations in the range favorable for the solution formation of HAP. In contrast, the release of PO_4 from both types of hybridized composites leveled off at considerably lower concentrations after only 24 h of immersion.

Mechanical Strength of Hybrid ACP-Filled BTHZ and TP Composites

Results of BFS testing of dry (before immersion) and wet (after the immersion in saline solutions) ACP/BTHZ and ACP/TP composites are summarized in Table VII. Generally, composite disks made of both hybridized as well as unhybridized ACP fillers had consistently lower *BFS* than the unfilled BTHZ or TP specimens. The strength of BTHZ composites was not significantly affected by the immersion process. However, the strength of the wet TP composites deteriorated significantly ($p \leq 0.0002$; unpaired *t*-test) upon prolonged immersion. Comparison of the *BFS* values of hy-

TABLE IV. Elemental Analysis of the Fillers[a]

Filler	Ca/PO_4 (Mol Ratio)	Si in the Solid (Mass Fraction, %)	Zr in the Solid (Mass Fraction, %)
Unhybridized	1.437 $\pm$ 0.119 (10)	n/a	n/a
5T	1.434 $\pm$ 0.079 (5)	2.74 $\pm$ 0.13 (3)	n/a
10T	1.476 $\pm$ 0.141 (5)	3.63 $\pm$ 0.18 (3)	n/a
20T	1.398 $\pm$ 0.039 (5)	2.82 $\pm$ 0.17 (3)	n/a
5Zr	1.550 $\pm$ 0.101 (5)	n/a	12.90 $\pm$ 0.38 (3)
10Zr	1.823 $\pm$ 0.123 (9)	n/a	8.58 $\pm$ 1.43 (3)
20Zr	1.901 $\pm$ 0.075 (5)	n/a	1.92 $\pm$ 0.14 (3)

[a]Results are given as the average value $\pm$ standard deviation. Number in parenthesis represents the number of samples in each group.

TABLE V. Specific Surface Area (SSA) of the Fillers[a]

Filler	SSA (m^2/g)
Unhybridized ACP	18.60 ± 1.16
5T-ACP	39.15 ± 2.87
10T-ACP	39.45 ± 0.73
20T-ACP	39.39 ± 1.56
5Zr-ACP	35.75 ± 3.97
10Zr-ACP	35.16 ± 3.86
20Zr-ACP	39.34 ± 4.30

[a]Results are given as the average value ± standard deviation of 3 independent runs in each experimental group.

bridized vs. unhybridized ACP/BTHZ composites showed that the *BFS* values of the hybridized specimens increased by similar percentages regardless of the type and initial amount of hybridizer. These relative increases in strength were 25–36% (dry samples) and 13–27% (wet samples) for TEOS-hybridized BTHZ composites, and 20–25% (dry samples) and 18–34% (wet samples) for $ZrOCl_2$-hybridized BTHZ composites. Dry TP composites also showed an improvement in mechanical strength (21–30% for TEOS and 21–28% for

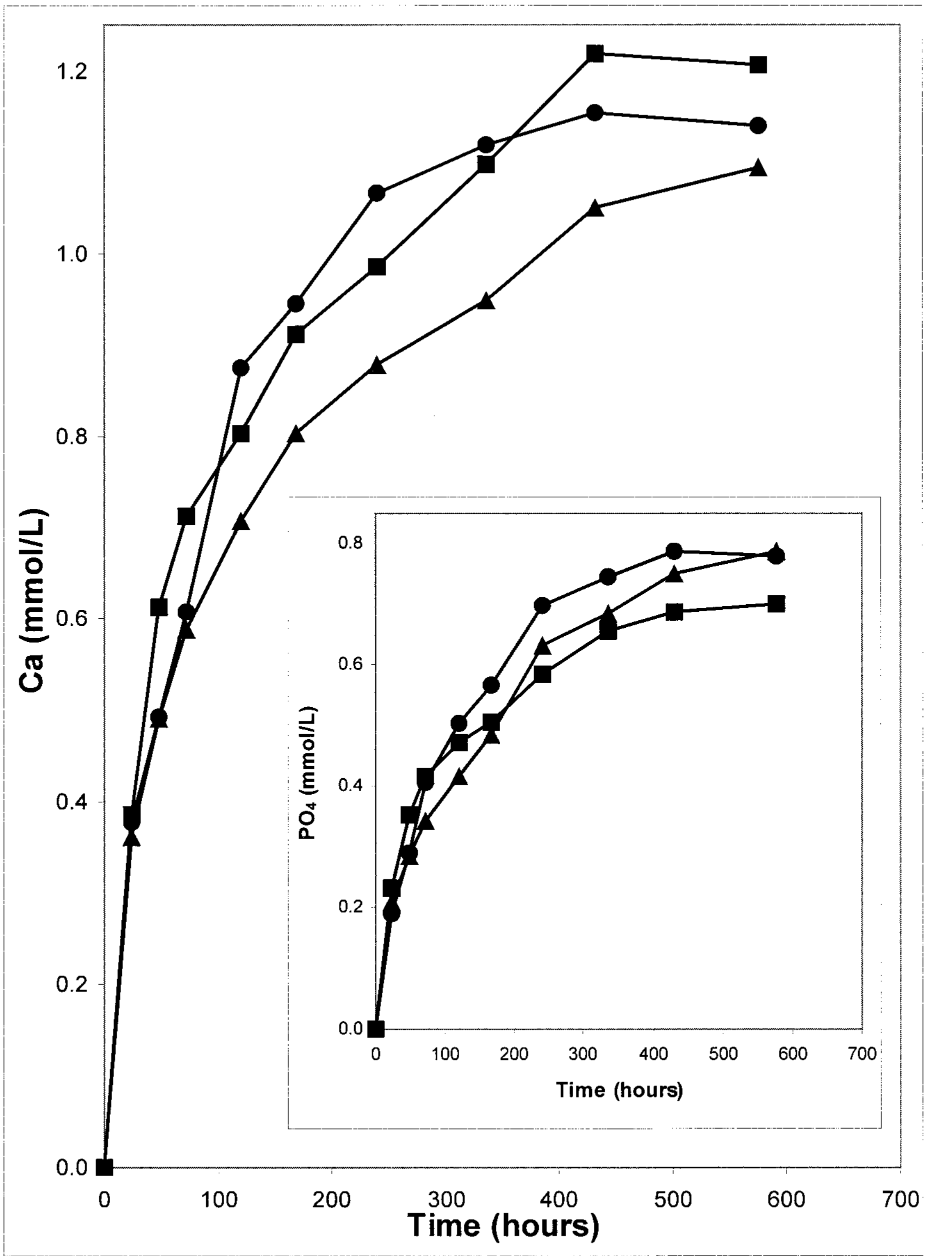

Figure 4. Ca^{2+} and PO_4 (inlet) ion release from the (■) 10T-, (▲) 10Zr-, and (●) unhybridized ACP BTHZ composites. Number of specimens: n = 5 for 10T-ACP, n = 8 for 10Zr-, and n = 12 for unhybridized ACP composites. Standard deviations of the plotted values were ≤ 0.137 mmol/L (Ca), ≤ 0.084 mmol/L (PO_4) for 10T-, ≤ 0.087 mmol/L (Ca), ≤ 0.073 mmol/L (PO_4) for 10Zr-, and ≤ 0.118 mmol/L (Ca), ≤ 0.075 mmol/L (PO_4) for unhybridized ACP composites.

TABLE VI. Average Values ± Standard Deviation of the Maximum Concentration of the Mineral Ions (Plateau Levels) Released from the Unhybridized and Hybridized BTHZ Composite Disks After >336 h of Immersion in Buffered Saline Solutions

Filler	No. of Runs	Ca (mmol/L)	PO_4 (mmol/L)
Unhybridized ACP	12	1.119 ± 0.118	0.752 ± 0.075
5T-ACP	5	1.072 ± 0.145	0.645 ± 0.070
10T-ACP	5	1.127 ± 0.137	0.656 ± 0.084
20T-ACP	8	1.242 ± 0.169	0.842 ± 0.074
5Zr-ACP	5	1.161 ± 0.118	0.748 ± 0.088
10Zr-ACP	8	0.993 ± 0.087	0.713 ± 0.073
20Zr-ACP	5	1.095 ± 0.148	0.698 ± 0.071

$ZrOCl_2$ composites). However, TP composites failed to maintain that strength upon immersion.

DISCUSSION

Hybridized ACP fillers synthesized in the presence of TEOS or $ZrOCl_2$ showed no differences in either long-range structural arrangements (XRD patterns) or local PO_4 environments (FTIR spectra). Elemental analysis (Ca, PO_4, Si, or Zr contents of the solids; Table IV) revealed some compositional differences, however. Mass fraction of approximately 3% of Si was incorporated (AAS analysis) and the Ca/PO_4 mol ratio was essentially constant in TEOS-hybridized fillers regardless of the amount of TEOS introduced *ab initio*. $ZrOCl_2$-hybridized ACP fillers, on the other hand, contained mass fraction of 2–13% Zr, with the amount of Zr incorporated being inversely proportional to the amount of hybridizer initially introduced during the ACP synthesis (linear dependence; correlation coefficient = -0.9977). The Ca/PO_4 mol ratios of Zr-ACPs, in contrast, showed the opposite trend; increasing with the level of hybridizer added. Findings that the specific surface areas (SSAs) of all hybridized solids investigated were considerably higher than the SSA of the unhybridized ACP (Table V) may be explained by the altered mode of ACP particle agglomeration in the presence of hybridizers. The higher SSA may also account for the observed increase in the mechanical strength of the composites. The constancy of the SSA (Table V) and the mass fraction (%) of Si (Table IV) in the hybridized ACP fillers suggest that the TEOS was not incorporated into the interior of the filler particles, but instead was bound on the surface of the particles. Excess TEOS was probably removed during filtering and washing of the freshly precipitated hybridized solids. The reason for the inverse proportionality between Zr in the solids and $ZrOCl_2$ in the preparative solution of $ZrOCl_2$-hybridized ACP fillers is not apparent. Possibly, at the higher solution concentrations, the $ZrOCl_2$ reacted with the Ca^{2+} to form a separate amorphous CaZrO complex that was removed during washing of the precipitated solids.

We have previously reported[3,4] that $P_2O_7^{4-}$-stabilized ACP was the most effective filler for establishing elevated Ca^{2+} and PO_4 ion concentrations upon soaking methacrylate-based polymeric composite disks in buffered saline solutions. For this reason, $P_2O_7^{4-}$-stabilized ACP was proposed as the most suitable of the ACP fillers for use in dental applications, where protection against mineral loss and/or restoration of mineral would be desirable.[4,5] Immersion of composites made of silica- or zirconia-hybridized, $P_2O_7^{4-}$-stabilized ACP

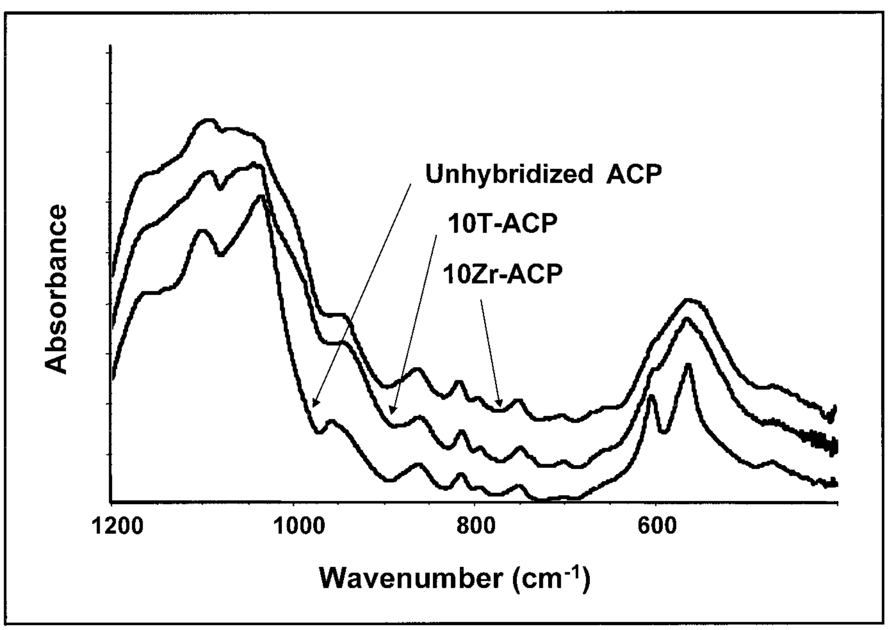

Figure 5. FTIR spectra (ground disk specimen; KBr pellets) of unhybridized-, 10T-, and 10Zr-ACP BTHZ composites exposed to the saline solutions for 576 h.

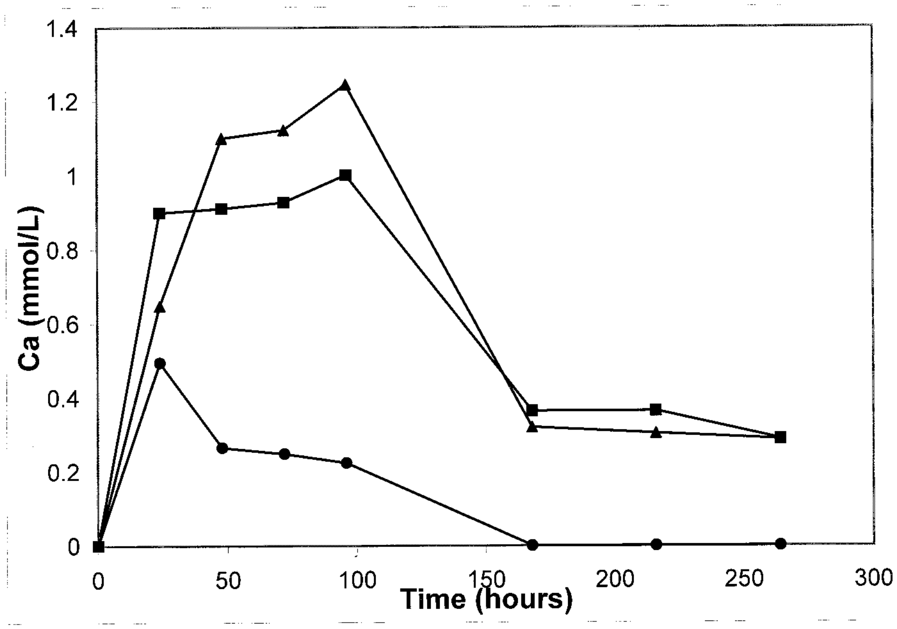

Figure 6. Average Ca^{2+} ion release from the (■) 10T-, (▲) 10Zr-, and (●) unhybridized ACP TP composites (triplicate experiments). Standard deviations of the plotted values were: ≤ 0.028 mmol/L (unhybridized ACP), ≤ 0.029 mmol/L (10T-ACP), and ≤ 0.044 mmol/L (Zr-ACP).

filler (used at optimal levels of 40% by mass) and BTHZ resin, also resulted in elevated mineral ion releases requisite for remineralization to occur. However, similarly treated composites made with TP resin failed to maintain a favorable remineralization potential due to the reincorporation by the composite of released Ca^{2+} and reduced PO_4 release. Observed Ca^{2+} consumption in TP composites can possibly be explained by the strong affinity of the carboxylic groups of PMGDM to bind ionic Ca^{2+}. Ca-PMGDM binding was indeed confirmed in a series of experiments, where disks made of unfilled TP resin were exposed to buffered saline solutions that initially contained 1.5 mmol/L Ca^{2+} (Fig. 8). The average observed drop in Ca^{2+} concentration of (0.63 ± 0.01) mmol/L correlates well with the Ca^{2+} decreases from rein-

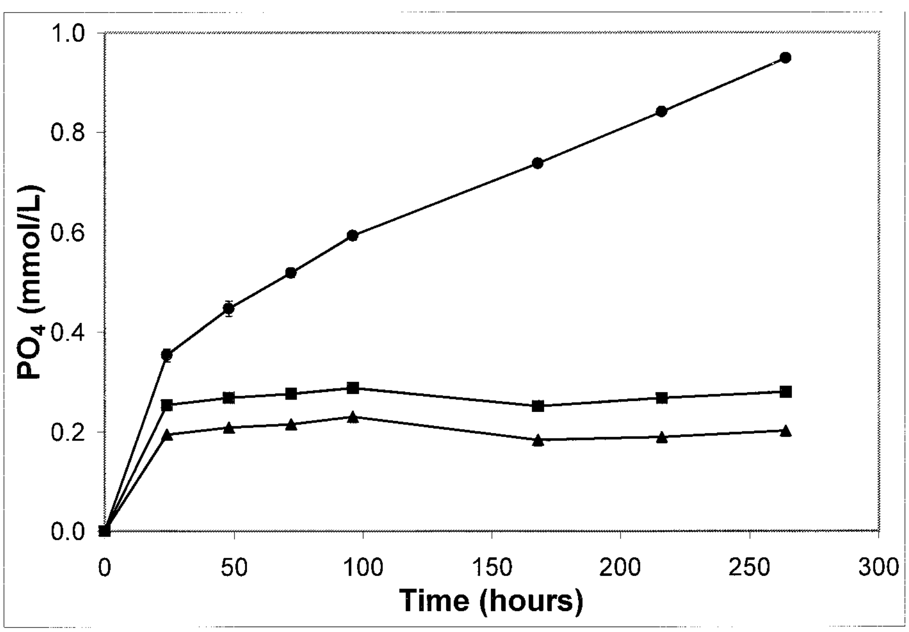

Figure 7. Average PO_4 ion release from the (■) 10T-, (▲) 10Zr-, and (●) unhybridized ACP TP composites (triplicate experiments). Standard deviations of the plotted values were: ≤ 0.015 mmol/L (unhybridized ACP), ≤ 0.014 mmol/L (10T-ACP), and ≤ 0.010 mmol/L (Zr-ACP).

TABLE VII. Biaxial Flexure Strength (BFS) of Unfilled and ACP-Filled BTHZ and TP Composites[a]

Resin Type	BFS (MPa); Dry Samples	BFS (MPa); Wet Samples
Unfilled BTHZ	107 ± 22 (21)[b]	100 ± 15 (7)
Unfilled TP	164 ± 31 (5)	101 ± 29 (10)
Unhybridized ACP-BTHZ	56 ± 8 (23)	56 ± 11 (35)
5T-ACP-BTHZ	70 ± 7 (5)	63 ± 4 (3)
10T-ACP-BTHZ	76 ± 9 (4)	65 ± 7 (3)
20T-ACP-BTHZ	72 ± 14 (13)	71 ± 13 (10)
5Zr-ACP-BTHZ	69 ± 10 (4)	66 ± 10 (3)
10Zr-ACP-BTHZ	70 ± 11 (17)	67 ± 10 (13)
20Zr-ACP-BTHZ	67 ± 6 (5)	75 ± 9 (3)
Unhybridized ACP-TP	61 ± 10 (5)	36 ± 7 (5)
5T-ACP-TP	79 ± 11 (6)	48 ± 10 (7)
10T-ACP-TP	74 ± 8 (6)	49 ± 7 (6)
20T-ACP-TP	77 ± 4 (6)	26 ± 2 (5)
5Zr-ACP-TP	78 ± 8 (6)	28 ± 4 (5)
10Zr-ACP-TP	74 ± 7 (6)	17 ± 1 (5)
20Zr-ACP-TP	75 ± 8 (6)	17 ± 5 (3)

[a]Comparison of unhybridized vs. hybridized ACP composites under dry (before immersion) and wet (after immersion in buffered saline solutions; immersion time 576 h).
[b]Values are given as mean value ± standard deviation with the number in parentheses indicating the number of specimens tested in each group.

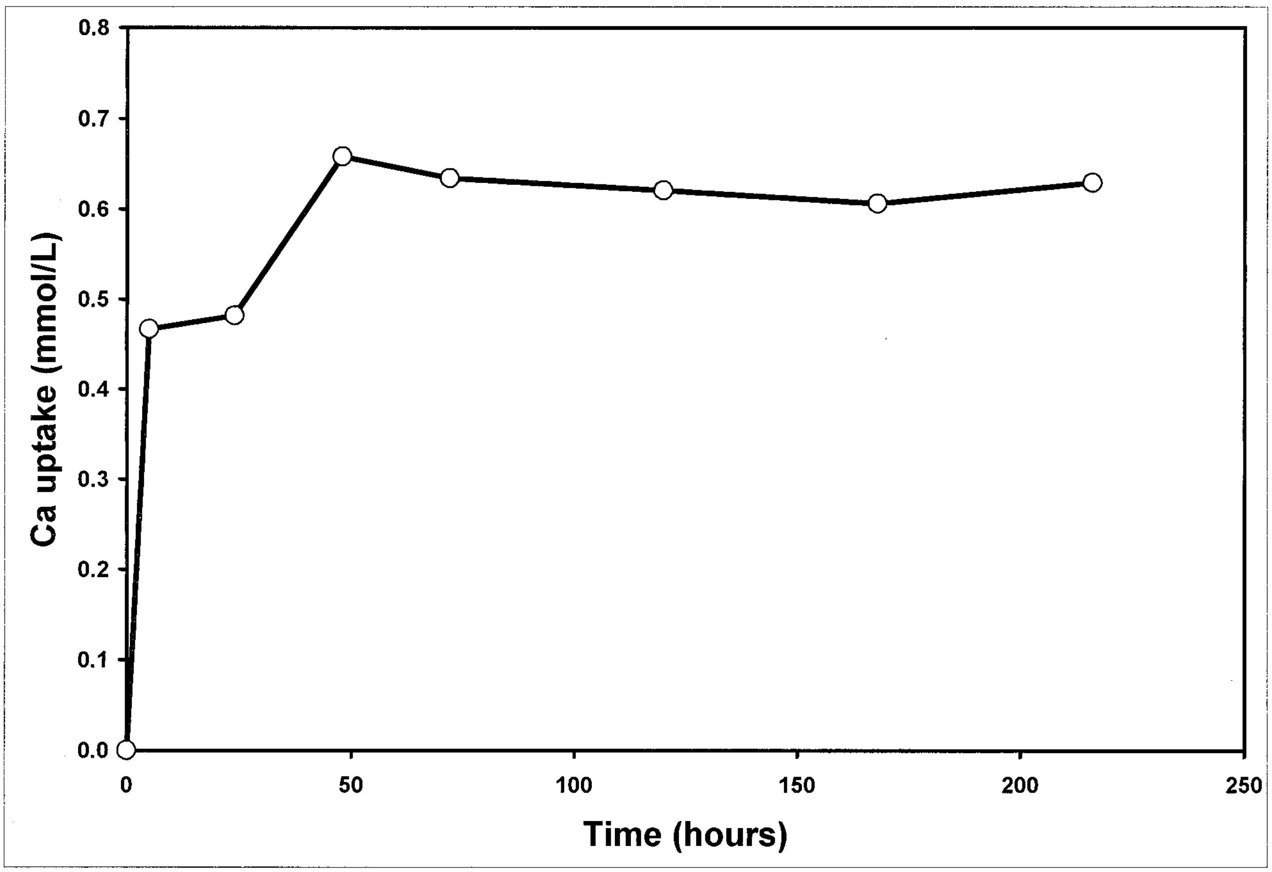

Figure 8. Kinetics of the Ca^{2+} uptake observed upon immersion of unfilled TP resin composite disk specimens in 100 mL of buffered saline solutions that initially contained 1.5 mmol/L Ca^{2+}. Given values are the averages of 3 independent runs. Standard deviations of the plotted values were ≤ 0.016 mmol/L.

corporation seen in both types of hybrid ACP/TP composites (0.56 ± 0.04) mmol/L and (0.75 ± 0.06) mmol/L for TEOS- and Zr-ACPs, respectively). Reincorporation also explains why no measurable Ca^{2+} was detected in the immersing solutions of unhybridized ACP/TP composites at time intervals > 150 h.

Unhybridized as well as hybridized ACP fillers formulated with either BTHZ or TP resins yielded polymerized materials weaker than either of the two unfilled polymers (Table VII). However, compared to composites with unhybridized fillers, mechanical strength of both wet and dry BTHZ composites that utilized hybridized fillers was enhanced, indicating improved filler-polymer interactions. The observed improvement in the strength of these hybridized composites probably resulted from better mechanical integration of hybrid fillers with the polymerized BTHZ resin. Hybridization of $P_2O_7^{4-}$-stabilized ACP is also more effective in making the experimental composites stronger than our previously described attempt to[12] chemically bind the P_2O_7-stabilized ACP filler and BTHZ polymer matrix with the silane coupling agent, 2-methacryloxypropyl trimethoxy silane. Although still inadequate to make these composites acceptable as bulk restoratives, the achieved strength enhancement may make hybridized ACP based BTHZ composites useful as pit and fissure sealants, or in similar applications, where the ability to prevent and/or redress tooth mineral loss is more important than high strength. TP composites, as currently formulated, cannot be considered for the same type of dental applications due to their inadequate mineral ion release and deteriorating strength upon immersion.

In conclusion, the results of this investigation demonstrate the possibility of enhancing the mechanical properties of ACP-filled BTHZ composites, while retaining their high remineralizing potential by introducing silica or zirconia elements during low-temperature synthesis of the ACP. Hybridized ACP fillers may extend the range of their applications as dental materials. Future studies will focus on the interactions of these hybrid ACP fillers with coupling agents and how the surface treatments affect the dissolution and mechanical behavior of such bioactive composites. Additionally, varying the chemical structure of the monomers and the composition of the resin phase of hybrid ACP composites will be systematically examined to establish the extent that changes in these parameters can influence their physicochemical, e.g., curability, hydrophilicity, ion release, and mechanical properties.

Disclaimer

Certain commercial materials and equipment are identified in this work for adequate definition of the experimental procedures. In no instance does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or that the material and the equipment identified are necessarily the best available for the purpose.

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